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ON HETEROTROPHIC NUTRITION OF SOME SPECIES OF GREEN ALGAE

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Introduction

Algae are mostly autotrophic, but there are species able to use organic compounds as a source of carbon or nitrogen. The favourable influence of organic substances upon algal growth was observed already by Beijerinck (1893) who succeeded in obtaining probably the first axenic algal cultures. Mendrecka (1913) pointed out that algal growth was favourably influenced especially by addition of carbchydrates and Beijerinck (1898) and Dangeard (1921) found that in this case some species were able to grow in the dark. The ability of heterotrophy in algae was later confirmed by Emerson (1927), Barker (1936), Kimball et al. (1963), Fournier (1966), and Ukeles and Rose (1976). Afterwards, the amplification of heterotrophy by light was discovered (Killam and Myers 1956, Pringsheim 1958, Samejima and Myers 1958, Mineeva 1962, Dvořakova-Hladka 1966, Karlender and Krauss 1966), and this type of nutrition was termed photoheterotrophy or mixotrophy.

The heterotrophy in algae was experimentally investigated mainly in liquid media. Wiedeman and Bold (1965) investigated the heterotrophy of algae on agar media but they did not follow it quantitatively. Quantitative data about the autotrophic growth of *Chlorella kesleri* on an agar medium were reported by Lukavský (1975).

During investigations of heterotrophy in various species and strains of *Chlorella* genus the formation of giant cells was described by several authors (Finkle et al. 1950, Oswald et al. 1953, Griffiths 1963, Andreeva 1967). Such a phenomenon in *Euglena gracilis* cells was observed by Maybelle et al. (1974).

The aim of this study was to determine the influence of various organic substances on the growth of cells and populations of algae in the light as well as in the dark.

Materials and Methods

Eight species of green algae were used in experiments: *Chlorella vulgaris* Beijer., *Scenedesmus quadricauda* (Turp.) Bréb., *Ankistrodesmus fusiformis* Corda et Korš., and *Ulothrix sp.* were isolated from the river Kupa; *Chlamydomonas peterfii* Gerl. was isolated from Dubravica peat-bog near Zagreb. The cultures of three species: *Chlamydomonas reinhardtii* Dang. 11/32-wild type, *Oocystis sp.* and *Chlorella sp.* (seawater species) were kindly supplied by Dr E. Marčenko, Ruđer Bošković Institute, Zagreb.

Axenic cultures of the above mentioned freshwater species were maintained in inorganic CHU-10 medium (Stein 1973) and seawater species Chlorella sp. in ASP-2 medium (Provasoli et al. 1957).

Organic components were added to the basal inorganic medium in the concentration of 0.05 M (pepton and yeast extract were used in the concentration of 1 per cent). Sixteen organic substances were used: d-glucose, d-fructose, d-galactose, d-mannose, d-ribose, d-lactose, d-maltose, d-raffinose, ethanol, Na-acetate, glycine, asparagine, urea, Bacto--peptone (Difco) and Bacto-yeast extract (Difco). The pH value of media was adjusted to 6.5—6.8 by means of TRIS-buffer or by means of NaOH solution in substrates with acetate. In the seawater media the pH value was adjusted to 8. The media were solidified with 1.5 per cent agar and then autoclaved (ethanol was added after sterilization). The plates were inoculated with about 50 cells (the plating efficiency was 60—100 per cent). Plating of the filamentous alga *Ulothrix* sp. was preceded by vigorous shaking of the primary culture in liquid medium resulting in the breaking off of long filaments into many very short segments. Before inoculation, the primary cultures were at the beginning of the stationary growth phase. Inoculated plates were placed in the light and in the dark. In the light the culture were maintained at 26 ± 0.5° C under fluorescent tubes (IPR, 40W, 6500° K, the daily regime of 16 h light and 8 h dark). The cultures in the dark were kept at the same temperature. After 12 days of incubation (exponential growth phase of colonies lasted about 10 days) the growth of colonies was established. The diameters of 20 to 30 colonies were measured under the stereomicroscope. The dimensions of the optical cell sections (20—30 cells) were also measured. With the help of photomicrographs and data concerning the optical cell sections the mean dimensions were estimated and the models of sections drawn on millimetre paper. The areas of optical cell sections, termed "optical cell section morphometric values", were determined by using the method of Weibel (1969). Most experiments were performed twice in duplicate.

Results and Discussion

The colony size after 12 days of incubation varied according to the growth substrate and to the presence or absence of light (Figs. 1—8). The growth was stimulated by the majority of organic substrates used and we can correlate it with heterotrophic nutrition. In some cases a favourable influence of light (photoheterotrophy) was observed. According to the stimulation (++++), inhibition (—) or neutral influence (+) of

substrates in the light (L) or in the dark (D), five types of colony growth could be differentiated:

	D	L
type 1	++++	++++
type 2	++++	+/—
type 3	+/—	++++
type 4	+	+
type 5	—	—

The substrates are classified according to the growth stimulating effect (according to the colony diameters) (Table 1). Four organic substances: glucose, fructose, peptone and yeast extract were found to be the most favourable substrates for algal growth. The ability of heterotrophy in the species compared is shown by the number of substrates which stimulated their growth (Table 2). The best heterotrophic (stimulated) growth was shown by *Scenedesmus quadricauda* and *Chlorella sp.* and the poorest by the filamentous alga *Ulothrix sp.* Several of the organic substrates stimulated growth in the dark; this can be seen by the unequal (unsymmetric) number of + signs in Table 1.

The heterotrophy in our strain of *Scenedesmus quadricauda* was confirmed by the experiment where the cells were able to devide and grow after 20 days of staying in dark. In this experiment quick transfer of cells to fresh medium was performed under deep green light.

Along with experiments where algal heterotrophy was investigated in liquid cultures, Wiedeman and Bold (1965) investigated the heterotrophy on agar media but did not follow the growth quantitatively, and Lukavský (1975) performed measurements of colony dimensions but only during autotrophic growth. Lukavský measured the diameter and the height of colonies, and in the present study only the diameter of colonies was measured. The growth of algae on organic substrates would probably be enhanced even more if some different concentrations and different light conditions were used. The favourable influence of peptone and yeast extract on the growth of algal colonies indicates the high nutritional value of their components for some algae.

The formation of enlarged and giant cells by all unicellular species grown on organic substrates were frequently observed (Table 3). Only the filamentous alga *Ulothrix* sp. showed no such change. The giant cells were most frequently observed in *Chlorella vulgaris* and *Oocystis sp.* Sometimes their sizes reached very high value as in the case of *Chlorella vulgaris* grown on lactose (in the light) where the optical cell sections were as much as seven times greater than the sections of cells grown in the inorganic control medium. There are several theories on the formation of giant cells in unicellular algae. Buchanan and Fulmer (1930) defined this phenomenon as the chemotrophic endeavour for survival in unfavourable life conditions. Griffiths (1963) ascribed the giant cell formation to heterotrophy as a dominant way of nutrition during the inhibition of cell division, and Maybelle et al. (1974) described them as a result of degenerative processes. Marčenko (1966) showed that gama radiation also caused the formation of giant cells in the green alga *Netrium digitus.* In the present study, the giant cell formation is considered to be the consequence of a disharmony between the process of cell growth and cell division.

Fig. 1—8. Effect of organic substances added to the inorganic (CHU-10 or ASP-2) medium on growth of: *Chlorella vulgaris* (Fig. 1), *Chlorella* sp. (Fig. 2), *Scenedesmus quadricauda* (Fig. 3), *Oocystis* sp. (Fig. 4), *Chlamydomonas reinhardtii* (Fig. 5), *Chlamydomonas peterfii* (Fig. 6), *Ankistrodesmus fusiformis* (Fig. 7), and *Ulothrix* sp. (Fig. 8). The height of the columns represents diameters of colonies after 12 days growth. Standard deviations are shown ($\bar{x} \pm s\bar{x}$).

Sl 1—8 Efekt organskih tvari dodanih anorganskom (CHU-10 ili ASP-2) mediju na rast kolonija vrsta: *Chlorella vulgaris* (Sl. 1), *Chlorella* sp. (Sl. 2), *Scenedesmus quadricauda* (Sl. 3), *Oocystis* sp. (Sl. 4), *Chlamydomonas reinhardtii* (Sl. 5), *Chlamydomonas peterfii* (Sl. 6), *Ankistrodesmus fusiformis* (Sl. 7) i *Ulothrix* sp. (Sl. 8). Visina stupca prikazuje promjer kolonija nakon 12 dana rasta. Prikazana je vrijednost standardne devijacije ($\bar{x} \pm s\bar{x}$).

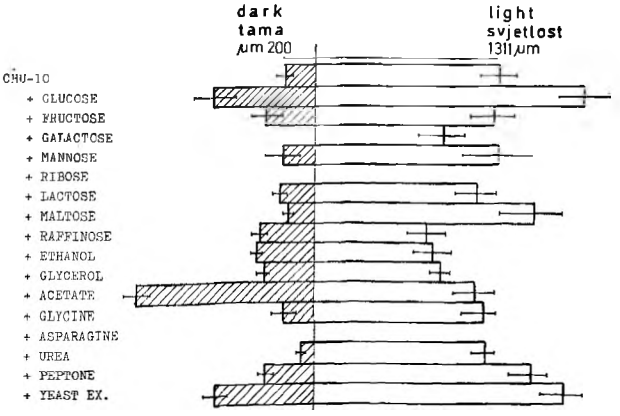
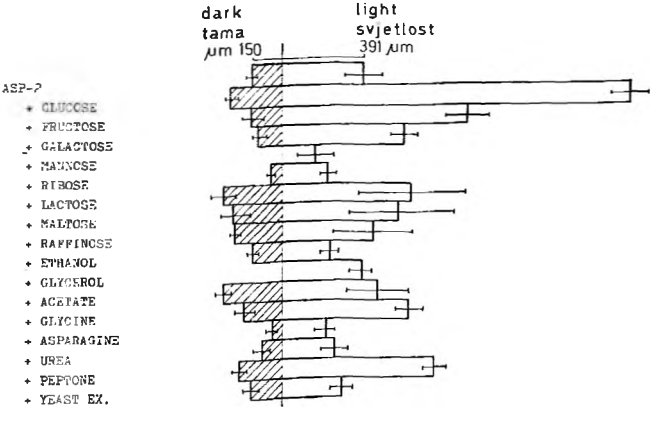


Fig. 1. — Sl. 1.



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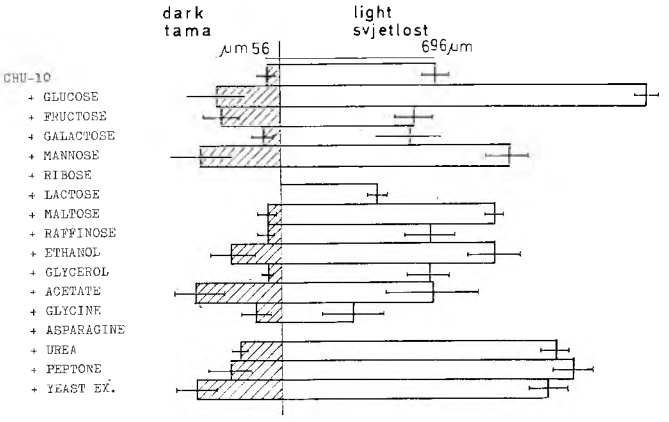
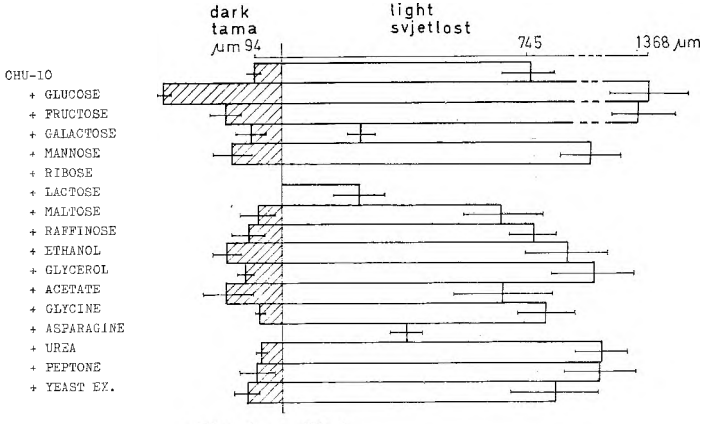


Fig. 3. — Sl. 3.



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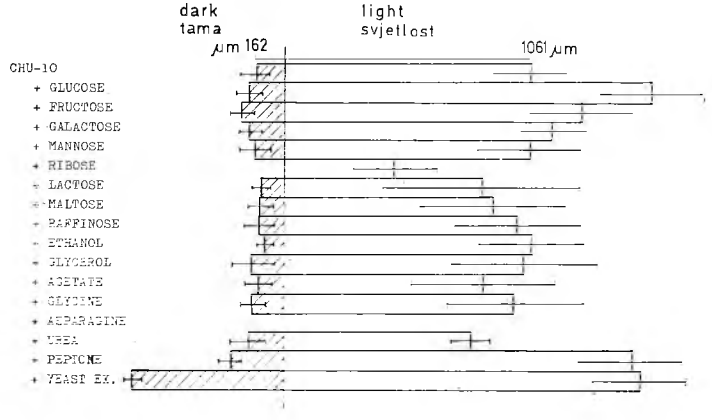

Fig. 5. — Sl. 5.

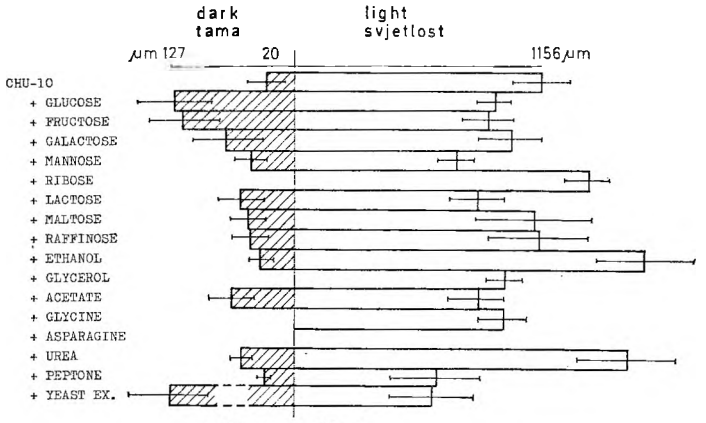

Fig. 6. — Sl. 6.

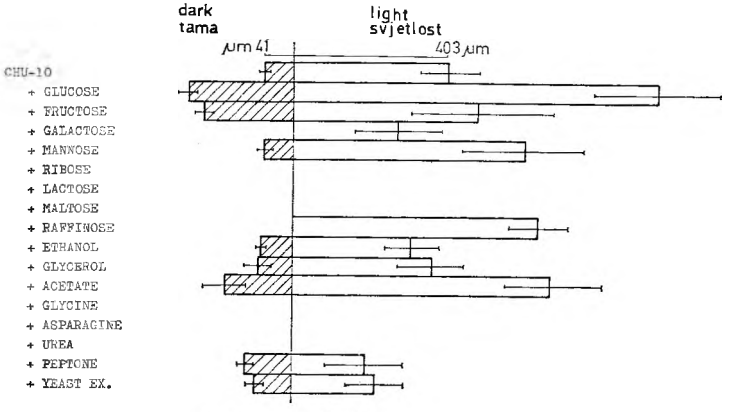

Fig. 7. — Sl. 7.

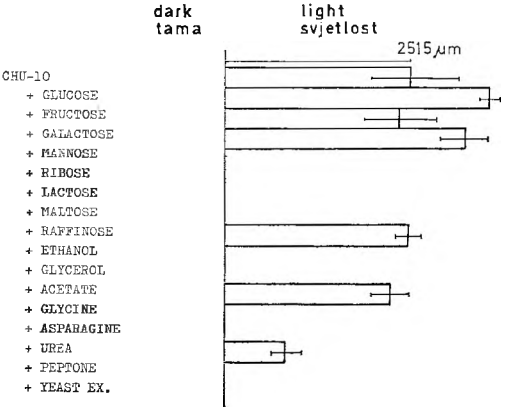

Fig. 8. — Sl. 8.

Table 1. Classification of substrates according to the number of species with stimulated growth of colonies. Stimulated growth refers to the dimension which was at least 10 per cent greater than the average control colony.

Tabela 1. Klasifikacija supstrata prema broju vrsta sa stimuliranim rastom kolonija. Rast je nazvan stimuliranim ako su kolonije bile za najmanje 10% veće od srednje veličine kolonija u kontroli.

(ASP−2)	dark tama	light svjetlost
CHU—10 + GLUCOSE	+++++++	+++++++
+ PEPTONE	+++++++	+++++
+ FRUCTOSE	+++++++	++++
+ YEAST EX.	+++++	++++
+ MANNOSE	++++	+++
+ ETHANOL	++++	+++
+ ACETATE	+++	+++
+ MALTOSE	++	+++
+ GLYCINE	+++++	++
+ RAFFINOSE	+++	++
+ GALACTOSE	+++	++
+ GLYCEROL	+++	+
+ ASPARAGINE	++	+
+ LACTOSE	++	+
+ RIBOSE		+
+ UREA		

Table 2. Classification of algae according to the ability of heterotrophy in the light and in the dark, expressed by the number of growth stimulating substrates. Stimulated growth refers to dimension which was at least 10 per cent greater than the average control colony.

Tabela 2. Klasifikacija alga prema sposobnosti heterotrofije na svjetlosti i u tami, izražena pomoću broja supstrata koji stimuliraju rast kolonija. Rast je nazvan stimuliranim, ako su kolonije bile za najmanje 10% veće od srednje veličine kolonija u kontroli.

dark tama		light svjetlost
+++++++++	*Chlorella* sp.	+++++++++
+++++++++	*Scenedesmus quadricauda*	+++++++
+++++++++++	*Chlamydomonas peterfii*	+++
++++++	*Oocystis* sp.	+++++++++
+++++++++	*Chlorella vulgaris*	++++
++++++++	*Chlamydomonas reinhardtii*	++++
++++++	*Ankistrodesmus fusiformis*	+++++
	Ulothrix sp.	++

Table 3. The relative »optical cell section morphometric values« of cells grown in the light (L) and in the dark (D). For the control medium the absolute morphometric value in brackets is specified. Double and higher values are in bold-faced type.

Tabela 3. Relativne veličine »morfometrijskih vrijednost :optičkih presjeka stanica« raslih na svjetlosti (L) i u tami (D). Za kontrolni medij navedena je u zagradi apsolutna morfometrijska vrijednost. Dvostruke i veće vrijednosti su istaknute.

	D	L	D	L	D	L	D	L	D	L	D	L	D	L
ASP−2	—	—	1(18)	1(18)	—	—	—	—	—	—	—	—	—	—
CHU−10	1(43)	1(29)	—	—	1(30)	1(26)	1(20)	1(29)	1(87)	1(78)	1(35)	1(54)	1(82)	1(58)
+ GLUCOSE	**4.37**	**2.58**	1.44	0.94	0.76	**2.38**	1.75	1.26	0.66	0.76	1.34	1.01	0.40	1.37
+ FRUCTOSE	**4.13**	1.44	1.77	0.94	0.83	1.19	1.25	1.17	0.51	0.89	1.17	1.05	0.40	1.22
+ GALACTOSE	0	1.34	1.33	0.88	0.83	0.92	**2.20**	1.75	0	0.88	1.34	1.03	0.43	1.79
+ MANNOSE	**3.25**	**2.20**	0	**3.44**	1.20	**2.65**	**2.25**	1.79	1.27	1.17	1.02	0.94	0.80	1.41
+ RIBOSE	0	0	0	0.94	0	0	0	0	0	0	0	1.77	0	1.67
+ LACTOSE	**7.63**	1.24	0.83	0.61	0	1.84	0	1.51	0	0	1.31	0.94	1.06	1.29
+ MALTOSE	**4.02**	1.72	0.88	0.77	1.30	1.84	**2.20**	0.89	0	0	1.14	1.11	0.90	1.00
+ RAFFINOSE	**4.11**	1.52	0.88	0.83	1.26	1.84	**2.61**	0.90	0	1.37	1.42	1.07	1.03	1.13
+ ETHANOL	**5.27**	1.82	0.50	0.77	0.83	1.92	**2.95**	**2.31**	1.24	1.42	1.42	1.07	0.85	1.08
+ GLYCEROL	**5.23**	1.24	0	0.66	1.63	1.57	**2.30**	0.82	0.79	1.48	1.28	1.12	0	1.63
+ GLYCINE	**6.83**	1.51	0.60	0.83	1.06	0.88	**2.21**	1.79	1.70	0.91	1.08	1.00	1.19	1.04
+ ASPARAGINE	**3.95**	1.44	0.94	0.77	0.83	1.11	1.80	1.37	0	0	1.00	1.03	0	1.63
+ UREA	0	**2.17**	0.88	0.88	0	0	0	1.51	0	0	0	0	0	0
+ ACETATE	1.34	1.06	0.88	1.22	1.32	1.96	**2.35**	1.68	0	0	1.37	1.57	1.22	1.37
+ PEPTONE	1.46	1.48	1.44	0.88	1.00	1.23	**2.25**	1.75	0.87	1.02	1.91	0.87	1.82	**2.05**
+ YEAST EX.	1.93	**2.96**	0.77	0.88	**2.43**	1.61	**2.90**	1.37	0.42	1.07	1.91	0.83	1.00	**2.03**
	Chlorella vulgaris		*Chlorella sp.*		*Scenedesmus quadricauda*		*Oocystis sp.*		*Ankistrodes. fusiformis*		*Chlamydomon. reinhardtii*		*Chlamydomon. peterfii*	

Summary

The growth of eight species of green algae on sixteen organic substrates in the light as well as in the dark was tested quantitatively. The growth was stimulated by most organic substrates and we can correlate it with heterotrophic nutrition. The heterotrophic growth was stimulated in the light. The best ability of heterotrophic growth has been shown in two species: *Scenedesmus quadricauda* and *Chlorella sp.*, and the poorest in *Ulothrix sp.* Glucose, fructose, peptone and yeast extract were the most efficient growth substrates and the least efficient were ribose and urea.

Enlarged and giant cells of unicellular species were usually present during their heterotrophic growth. These changes are considered to be the consequence of the disharmony between the process of cell growth and cell division.

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SAŽETAK

O HETEROTROFNOJ ISHRANI NEKIH VRSTA ZELENIH ALGA

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Kvantitativno je ispitivano rastenje osam vrsta zelenih alga na šesnaest organskih supstrata, kako na svjetlosti tako i u tami.

Mnogi organski supstrati stimulirali su rast, pa to možemo povezati sa heterotrofnom prehranom. Svjetlost je stimulirala heterotrofni rast. Najpogodniji supstrati za heterotrofiju bili su: glukoza, fruktoza, pepton i ekstrakt kvasca, a inhibiciju rastenja kod većine vrsta izazvali su riboza i urea. Najbolji heterotrofni rast pokazale su vrste *Scenedesmus quadricauda* i *Chlorella* sp., a najslabiji nitasta alga *Ulothrix* sp.

Kod jednostaničnih vrsta bile su često prisutne povećane i divovske stanice. Te promjene protumačene su kao poremećaj ravnoteže između procesa rastenja i dijeljenja stanica.

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